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Association of variations in the *FTO*, *SCG3*, and *MTMR9* genes with metabolic syndrome in a Japanese population

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ABSTRACT

Metabolic syndrome is defined as a cluster of multiple risk factors, including central obesity, dyslipidemia, hypertension, and impaired glucose tolerance, that increase cardiovascular disease morbidity and mortality. Genetic factors are important in the development of metabolic syndrome, as are environmental factors. However, the genetic background of metabolic syndrome is not yet fully clarified. There is evidence that obesity and obesity-related phenotypes are associated with variations in several genes, including *NEGR1*, *SEC16B*, *TMEM18*, *ETV5*, *GNPDA2*, *BDNF*, *MTCH2*, *SH2B1*, *FTO*, *MAF*, *MC4R*, *KCTD15*, *SCG3*, *MTMR9*, *TFAP2B*, *MSRA*, *LYPLAL1*, *GCKR*, and *FADS1*. To investigate the relationship between metabolic syndrome and variations in these genes in the Japanese population, we genotyped 33 single-nucleotide polymorphisms (SNPs) in 19 genes from 1096 patients with metabolic syndrome and 581 control individuals who had no risk factors for metabolic syndrome. Four SNPs in the *FTO* gene were significantly related to metabolic syndrome: rs9939609 ($P = 0.00013$), rs8050136 ($P = 0.00011$), rs1558902 ($P = 6.6 \times 10^{-5}$), and rs1421085 ($P = 7.4 \times 10^{-5}$). rs3764220 in the *SCG3* gene ($P = 0.0010$) and rs2293855 in the *MTMR9* gene ($P = 0.0015$) were also significantly associated with metabolic syndrome. SNPs in the *FTO*, *SCG3*, and *MTMR9* genes had no SNP \times SNP epistatic effects on metabolic syndrome. Our

data suggest that genetic variations in the *FTO*, *SCG3*, and *MTMR9* genes independently influence the risk of metabolic syndrome.

Key words: *FTO*, *SCG3*, *MTMR9*, metabolic syndrome

INTRODUCTION

Metabolic syndrome is a common clinical phenotype that is concurrent with metabolic abnormalities, including central obesity, glucose intolerance, dyslipidemia, and hypertension.¹ Several different definitions of the syndrome exist² and there are still debates on the adequacy of the concept; however, metabolic syndrome has attracted considerable interest. Although the pathogenesis of metabolic syndrome is not fully understood, the predominant underlying risk factor is considered to be central obesity due to an atherogenic diet and physical inactivity in the presence of some genetic background.^{2, 3} Adipose tissue, especially visceral fat, secretes various adipocytokines. An increase in adipose tissue mass leads to an alteration in the plasma levels of adipocytokines, resulting in the development of dyslipidemia, hypertension, and insulin resistance.^{3, 4}

Results from studies using twins and families have suggested that genetic and environmental factors contribute to the clustering of metabolic abnormalities in various ethnic groups.⁵⁻¹⁰ We determined in a previous study that 4 single-nucleotide polymorphisms (SNPs) (rs2294901, rs6133922, rs6077785, and rs6108572) in the McKusick–Kaufman syndrome (*MKKS*) gene were significantly associated with metabolic syndrome in the Japanese population¹¹ by screening SNPs in 85 obesity-related genes that had been reported as of 2005.¹² We carried out a large-scale case-control association study and found that secretogranin III

(*SCG3*)¹³ and myotubularin-related protein 9 (*MTMR9*)¹⁴ conferred susceptibility to the obesity phenotype in the Japanese population. Recent genome-wide association studies revealed the SNPs associated with obesity and fat distribution (waist circumference and waist to hip ratio).¹⁵⁻¹⁹ We confirmed that some of these SNPs are also associated with obesity and visceral fat area as determined by computed tomography (CT) in the Japanese population.²⁰⁻²²

In this study, we investigated the association between metabolic syndrome and 33 SNPs related to obesity and the obesity-related phenotype. We found that SNPs in the fat mass and obesity associated (*FTO*), *SCG3*, and *MTMR9* genes are associated with metabolic syndrome.

MATERIALS AND METHODS

Study subjects

The sample size of the group of Japanese subjects with metabolic syndrome was 1096 (male to female ratio, 594:502; age, 53.8 ± 12.5 years). The sample size of the group of Japanese controls was 581 (male to female ratio, 182:399; age, 47.2 ± 14.8 years). Metabolic syndrome was diagnosed as reported previously.^{11, 23} In brief, metabolic syndrome is defined by the presence of 2 or more metabolic abnormalities in addition to obesity (body mass index [BMI] > 25 kg m^{-2}). The metabolic abnormalities were as follows: (1) triglyceride level $\geq 150 \text{ mg per}$

100 ml and/or high-density lipoprotein cholesterol level < 40 mg per 100 ml, or under treatment for this type of dyslipidemia; (2) systolic blood pressure \geq 130 mm Hg and/or diastolic blood pressure \geq 85 mm Hg, or under treatment for hypertension; and (3) fasting glucose level \geq 110 mg per 100 ml, or under treatment for diabetes. The control group consisted of the subjects who were not obese ($\text{BMI} < 25 \text{ kg m}^{-2}$) and who exhibited none of the metabolic abnormalities described above. Clinical characteristics of subjects are summarized in Table 1. Subjects with metabolic syndrome were recruited from outpatient clinics. Control subjects were selected from non-obese Japanese volunteers who had undergone a medical examination for common disease screening. Written informed consent was obtained from each subject, and the protocol was approved by the ethics committee of each institution and by that of Kyoto University.

DNA preparation and SNP genotyping

Using Genomix (Talent Srl, Trieste, Italy), genomic DNA was extracted from blood samples collected from each subject. We constructed Invader probes (Third Wave Technologies, Madison, WI, USA) for rs3101336, rs2568958, and rs2815752 in the neuronal growth regulator 1 (*NEGR1*) gene; rs10913469 in the SEC16 homolog B (*SEC16B*) gene; rs6548238 and rs7561317 in the transmembrane protein 18 (*TMEM18*) gene; rs7647305 in the ets variant 5 (*ETV5*) gene; rs10938397 in the glucosamine-6-phosphate deaminase 2 (*GNPDA2*) gene;

rs6265 and rs925946 in the brain-derived neurotrophic factor (*BDNF*) gene; rs10838738 in the mitochondrial carrier homolog 2 (*MTCH2*) gene; rs7498665 in the SH2B adaptor protein 1 (*SH2B1*) gene; rs9939609, rs8050136, rs6499640, rs1121980, rs1558902, and rs1421085 in the *FTO* gene; rs1424233 in the v-maf musculo-aponeurotic fibrosarcoma oncogene homolog (*MAF*) gene; rs17782313, rs12970134, rs489693, and rs17700144 in the melanocortin 4 receptor (*MC4R*) gene; rs29941 and rs11084753 in the potassium channel tetramerisation domain containing 15 (*KCTD15*) gene; rs3764220 in the *SCG3* gene; rs2293855 in the *MTMR9* gene; rs987237 in the transcription factor AP-2 β (*TFAP2B*) gene; rs7826222 in the methionine sulfoxide reductase A (*MSRA*) gene; rs2605100 in the lysophospholipase-like-1 (*LYPLAL1*) gene; rs780094 and rs1260326 in the glucokinase regulator (*GCKR*) gene; and rs174547 in the fatty acid desaturase 1 (*FADS1*) gene. The SNPs were genotyped using Invader assays as previously described.²⁴ The success rate of these assays was > 99.0%.

Statistical analysis

For the additive model, we coded genotypes as 0, 1, or 2, depending on the number of copies of the risk alleles. For the dominant model, homozygosity and heterozygosity with the risk allele were coded as 1 and the other was coded as 0. For the recessive model, homozygosity with the risk allele was coded as 1 and others were coded as 0. Additive, dominant, and recessive models were chosen for each SNP, according to data from the previous reports¹³⁻²². Odds

ratios (ORs) and *P*-values adjusted for age and gender were calculated using multiple logistic regression analysis with genotypes, age, and gender as the independent variables. The Hardy–Weinberg equilibrium was assessed using the χ^2 -test.²⁵ Simple comparison of the clinical data between case and control groups was carried out using the Mann–Whitney *U*-test. To test SNP \times SNP epistasis for case–control population–based samples, we used the logistic regression model for each SNP1 and SNP2, and fits the model in the form of $Y = \beta_0 + \beta_1 \times \text{SNP1} + \beta_2 \times \text{SNP2} + \beta_3 \times \text{SNP1} \times \text{SNP2} + \beta_4 \times \text{age} + \beta_5 \times \text{gender}$. Statistical analysis was performed using the software R (<http://www.r-project.org/>). *P*-values were corrected by Bonferroni adjustment and $P < 0.00152$ ($0.05/33$) was considered significant.

We examined the power ($\alpha = 0.00152$) of the test for 1096 cases and 581 controls using GDesignPlus (StaGen Co Ltd., Tokyo, Japan). We considered the controls of 4 different levels of minor allele frequencies (MAF) (0.1–0.4) and 4 different levels of the ORs (1.2–1.5). Obtained power was indicated in Supplementary Table 1.

RESULTS

We used measurements of BMI ($>25 \text{ kg m}^{-2}$) instead of waist circumference, as there are still debates on the criteria for waist circumference, especially in Japanese women. The prevalence of metabolic syndrome increased with subject age and has been previously reported to be

approximately 6–7 times higher in men than in women in Japan.²³ Among our subjects, the ratio of men to women and the average age were both significantly higher in the case group than in the control group (Table 1). To adjust for the effects of age and gender, logistic regression analysis was performed. The most significant associations were observed for rs1558902 ($P = 6.6 \times 10^{-5}$, allele-specific OR (95% confidence interval (CI) adjusted for age and gender) = 1.47 (1.22–1.78)) and rs1421085 ($P = 7.4 \times 10^{-5}$, allele-specific OR (95% CI adjusted for age and gender) = 1.47 (1.21–1.77)) in the *FTO* gene (Table 2). These 2 SNPs were previously reported as variations associated with waist circumference^{18, 19} and associated with visceral fat area as determined by CT.²² Two other SNPs (rs9939609 and rs8050136) in the *FTO* gene were also significantly associated with metabolic syndrome even when the conservative Bonferroni's correction was applied ($P < 0.00152$). Significant associations were also observed between metabolic syndrome and rs3764220 in the *SCG3* gene ($P = 0.0010$) and rs2293855 in the *MTMR9* gene ($P = 0.00146$). SNPs, rs6548238 and rs7561317 in the *TMEM18* gene, rs7498665 in the *SH2B1* gene, and rs1121980 in the *FTO* gene, were marginally associated with metabolic syndrome ($P < 0.05$). SNP rs10838738 in the *MTCH2* gene was also marginally associated with metabolic syndrome, although presence of the G-allele, which is a risk allele for obesity,^{15, 16, 21} was associated with a reduced risk of metabolic syndrome. Other SNPs did not show any significant associations with metabolic syndrome. The lack of

significant association of these SNPs is most likely due to the relatively lower power of this study (Supplementary table 1). All SNPs were in Hardy–Weinberg equilibrium ($P > 0.05$), with the exception of rs6499640 ($P = 0.0099$) and rs1424233 ($P = 0.0034$) in the case group and rs3764220 in the control subjects ($P = 0.0018$).

Since 6 SNPs were significantly associated with metabolic syndrome, we tested SNP \times SNP epistasis. Four SNPs in the *FTO* gene were in a linkage disequilibrium (LD) ($r^2 > 0.97$), thus, pair of these SNPs were not analyzed. Any pairs of SNPs did not show significant epistatic effect on metabolic syndrome (Table 3). We performed the multiple logistic regression analysis with 3 genotypes (rs1558902, rs3764220, and rs2293855), age, and gender as the independent variables and found that effects of these SNPs in the three genes on metabolic syndrome were additive: rs1558902 (additive model), $P = 4.1 \times 10^{-5}$, OR (95% CI) = 1.50 (1.23 – 1.81); rs3764220 (dominant model), $P = 0.00018$, OR (95% CI) = 4.77 (2.11 – 10.79); rs2293855 (recessive model), $P = 0.00090$, OR (95% CI) = 1.46 (1.17 – 1.82). Similar results were obtained using rs9939609, rs8050136, and rs1421085, instead of rs1558902.

Next, we examined the effects of significant SNPs on each metabolic disorder (dyslipidemia, hypertension, and impaired fasting glucose). SNPs (rs9939609, rs8050136, rs1558902, and rs1421085) in the *FTO* gene were significantly associated with dyslipidemia, hypertension, and impaired fasting glucose (Table 4). rs2293855 in the *MTMR9* gene was

significantly associated with hypertension and impaired fasting glucose, and was marginally associated with dyslipidemia. rs3764220 in the *SCG3* gene was marginally associated with all 3 metabolic disorders. There were no obvious differences among the effects (OR) on each metabolic disorder.

DISCUSSION

The SNPs that were most significantly associated with metabolic syndrome (rs1558902 and rs1421085) exist in the *FTO* gene. Visceral fat accumulation is the most predominant factor for the development of metabolic syndrome. SNPs (rs1558902 and rs1421085) were reported to be associated with waist circumference and visceral fat area as measured by CT.^{18, 19, 22} SNPs (rs9939609, rs8050136, rs1558902 and rs1421085) were also associated with obesity and type 2 diabetes.¹⁵⁻¹⁷ We have previously reported that rs3764220 in the *SCG3* gene was associated with obesity and subcutaneous fat area as measured by CT¹³ and that rs2293855 in the *MTMR9* gene was associated with obesity and hypertension.¹⁴ Therefore, it is likely that rs9939609, rs8050136, rs1558902 and rs1421085 in the *FTO*, rs3764220 in the *SCG3* and rs2293855 in the *MTMR9* genes would be susceptible for metabolic syndrome. Epistasis, or gene–gene interaction, has recently received much attention in human genetics.²⁶ In this study, effect of these SNPs on metabolic syndrome was independent, and epistatic effect was not

observed.

In the simulation study, the power of this test was 0.317 in the following condition; the sizes of the case and the control groups are 1096 and 581, respectively, OR is 1.4, risk allele frequency is 0.2 and the model of inheritance is additive. Therefore, further studies would be necessary to elucidate the association between SNPs and metabolic syndrome.

SNPs (rs780094 and rs1260326) in the *GCKR* gene were previously reported to be associated with metabolic disorders²⁷⁻²⁹; however, the present study did not show the association with metabolic syndrome. The C-allele of rs780094 was reported to be associated with increased fasting plasma glucose and lower triglyceride levels. The risk allele of rs780094 had an antagonistic affect on plasma glucose and triglycerides levels. SNP rs174547 in the *FADS1* gene, which is in almost complete linkage disequilibrium with rs174550, is associated with dyslipidemia and type 2 diabetes.^{28, 30} Similar to rs780094, the effects of the risk allele of rs174547 on type 2 diabetes, hypertension, and dyslipidemia are not the same. Thus, rs780094 and rs174550 are not likely to be important in the development of metabolic syndrome.

SNPs (rs6548238 and rs7561317) in the *TMEM18* gene were marginally associated with metabolic syndrome. We have reported that these SNPs are associated with obesity.²¹ Takeuchi *et al.* reported that rs48454344, which is in almost complete linkage disequilibrium with rs6548238 and rs7561317,²¹ was associated with BMI and type 2 diabetes.³¹ Therefore,

rs6548238 and rs7561317 in the *TMEM18* gene might be susceptible for the development of metabolic syndrome.

The G-allele of rs10838738 in the *MTCH2* gene, which is a risk allele for obesity,¹⁶ reduced the risk of metabolic syndrome. Takeuchi *et al.* reported that the G-allele is a risk factor for obesity and is protective for type 2 diabetes.³¹ Although further investigations would be necessary, there is a possibility that there are some variations that are specifically associated with components of metabolic syndrome, apart from obesity.

Our approach effectively indicated that SNPs in the *FTO*, *SCG3*, and *MTMR9* genes are associated with metabolic syndrome in the Japanese population, in addition to the previously reported SNPs in the *MKKS* gene. SNPs in the *FTO*, *SCG3*, and *MTMR9* genes had no SNP × SNP epistatic effects on metabolic syndrome. Since metabolic syndrome is a complex concept and the criteria are still controversial, further investigations are necessary to elucidate the roles of SNPs in the *MKKS*, *FTO*, *SCG3*, and *MTMR9* genes in the development of metabolic syndrome.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERNECS

1. Carr, D. B., Utzschneider, K. M., Hull, R. L., Kodama, K., Retzlaff, B. M., Brunzell, J. D. *et al.* Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes* **53**, 2087–2094 (2004).
2. Pollex, R. L. & Hegele, R. A. Genetic determinants of the metabolic syndrome. *Nat. Clin. Pract. Cardiovasc. Med.* **3**, 482–489 (2006).
3. Matsuzawa, Y. Therapy insight: adipocytokines in metabolic syndrome and related cardiovascular disease. *Nat. Clin. Pract. Cardiovasc. Med.* **3**, 35–42 (2006).
4. Hotta, K., Funahashi, T., Bodkin, N. L., Ortmeier, H. K., Arita, Y., Hansen, B. C. *et al.* Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* **50**, 1126–1133 (2001).
5. Carmelli, D., Cardon, L. R. & Fabsitz, R. Clustering of hypertension, diabetes, and obesity

- in adult male twins: same genes or same environments? *Am.J.Hum.Genet.* **55**, 566–573 (1994).
6. Edwards, K. L., Newman, B., Mayer, E., Selby, J. V., Krauss, R. M. & Austin, M. A. Heritability of factors of the insulin resistance syndrome in women twins. *Genet. Epidemiol.* **14**, 241–253 (1997).
 7. Austin, M. A., Edwards, K. L., McNeely, M. J., Chandler, W. L., Leonetti, D. L., Talmud, P. J. *et al.* Heritability of multivariate factors of the metabolic syndrome in nondiabetic Japanese Americans. *Diabetes* **53**, 1166–1169 (2004).
 8. Chen, W., Srinivasan, S. R., Elkasabany, A. & Berenson, G. S. The association of cardiovascular risk factor clustering related to insulin resistance syndrome (Syndrome X) between young parents and their offspring: the Bogalusa Heart Study. *Atherosclerosis* **145**, 197–205 (1999).
 9. Lee, K. E., Klein, B. E. & Klein, R. Familial aggregation of components of the multiple metabolic syndrome in the Framingham Heart and Offspring Cohorts: Genetic Analysis Workshop Problem 1. *BMC Genet.* **4**, S94–S98 (2003).
 10. Wu, K. D., Hsiao, C. F., Ho, L. T., Sheu, W. H., Pei, D., Chuang, L. M. *et al.* Clustering and heritability of insulin resistance in Chinese and Japanese hypertensive families: a Stanford-Asian Pacific Program in Hypertension and Insulin Resistance Sibling Study.

Hypertens. Res. **25**, 529–536 (2002).

11. Hotta, K., Nakamura, T., Takasaki, J., Takahashi, H., Takahashi, A., Nakata, Y., *et al.*

Screening of 336 single-nucleotide polymorphisms in 85 obesity-related genes revealed

McKusick-Kaufman syndrome gene variants are associated with metabolic syndrome. *J.*

Hum. Genet. **54**, 230-235 (2009).
12. Rankinen, T., Zuberi, A., Chagnon, Y. C., Weisnagel, S. J., Argyropoulos, G., Walts, B. *et al.*

The human obesity gene map: the 2005 update. *Obesity* **14**, 529–644 (2006).
13. Tanabe, A., Yanagiya, T., Iida, A., Saito, S., Sekine, A., Takahashi, A. *et al.* Functional

single-nucleotide polymorphisms in the secretogranin III (SCG3) gene that form secretory

granules with appetite-related neuropeptides are associated with obesity. *J. Clin.*

Endocrinol. Metab. **92**, 1145–1154 (2007).
14. Yanagiya, T., Tanabe, A., Iida, A., Saito, S., Sekine, A., Takahashi, A. *et al.* Association of

single-nucleotide polymorphisms in MTMR9 gene with obesity. *Hum. Mol. Genet.* **16**,

3017–3026 (2007).
15. Thorleifsson, G., Walters, G. B., Gudbjartsson, D. F., Steinthorsdottir, V., Sulem, P.,

Helgadottir, A. *et al.* Genome-wide association yields new sequence variants at seven loci

that associate with measures of obesity. *Nat. Genet.* **41**, 18–24 (2009).
16. Willer, C. J., Speliotes, E. K., Loos, R. J., Li, S., Lindgren, C. M., Heid, I. M. *et al.* Six new

- loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat. Genet.* **41**, 25–34 (2009).
17. Meyre, D., Delplanque, J., Chèvre, J. C., Lecoœur, C., Lobbens, S., Gallina, S. *et al.* Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat. Genet.* **41**, 157–159 (2009).
18. Lindgren, C. M., Heid, I. M., Randall, J. C., Lamina, C., Steinthorsdottir, V., Qi, L. *et al.* Genome-wide association scan meta-analysis identifies three loci influencing adiposity and fat distribution. *PLoS Genet.* **5**, e1000508 (2009).
19. Heard-Costa, N. L., Zillikens, M. C., Monda, K. L., Johansson, A., Harris, T. B., Fu, M. *et al.* NRXN3 is a novel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. *PLoS Genet.* **5**, e1000539 (2009).
20. Hotta, K., Nakata, Y., Matsuo, T., Kamohara, S., Kotani, K., Komatsu, R. *et al.* Variations in the FTO gene are associated with severe obesity in the Japanese. *J. Hum. Genet.* **53**, 546–553 (2008).
21. Hotta, K., Nakamura, M., Nakamura, T., Matsuo, T., Nakata, Y., Kamohara, S. *et al.* Association between obesity and polymorphisms in SEC16B, TMEM18, GNPDA2, BDNF, FAIM2 and MC4R in a Japanese population. *J. Hum. Genet.* **54**, 727–731 (2009).
22. Hotta, K., Nakamura, M., Nakamura, T., Matsuo, T., Nakata, Y., Kamohara, S. *et al.*

- Polymorphisms in NRXN3, TFAP2B, MSRA, LYPLAL1, FTO and MC4R and their effect on visceral fat area in the Japanese population. *J. Hum. Genet.* **55**, 738–742 (2010).
23. Arai, H., Yamamoto, A., Matsuzawa, Y., Saito, Y., Yamada, N., Oikawa, S. *et al.* Prevalence of metabolic syndrome in the general Japanese population in 2000. *J. Atheroscler. Thromb.* **13**, 202–208 (2006).
24. Ohnishi, Y., Tanaka, T., Ozaki, K., Yamada, R., Suzuki, H. & Nakamura, Y. A high-throughput SNP typing system for genome-wide association studies. *J. Hum. Genet.* **46**, 471–477 (2001).
25. Nielsen, D. M., Ehm, M. G. & Weir, B. S. Detecting marker-disease association by testing for Hardy–Weinberg disequilibrium at a marker locus. *Am. J. Hum. Genet.* **63**, 1531–1540 (1998).
26. Cordell H. Detecting gene–gene interactions that underlie human diseases. *Nat. Rev. Genet.* **10**, 392–404 (2009).
27. Bi, M., Kao, W. H., Boerwinkle, E., Hoogeveen, R. C., Rasmussen-Torvik, L. J., Astor, B. C. *et al.* Association of rs780094 in GCKR with metabolic traits and incident diabetes and cardiovascular disease: the ARIC Study. *PLoS One* **5**, e11690 (2010).
28. Dupuis, J., Langenberg, C., Prokopenko, I., Saxena, R., Soranzo, N., Jackson, A. U. *et al.* New genetic loci implicated in fasting glucose homeostasis and their impact on type 2

- diabetes risk. *Nat. Genet.* **42**, 105-116 (2010).
29. Onuma, H., Tabara, Y., Kawamoto, R., Shimizu, I., Kawamura, R., Takata, Y. *et al.* The GCKR rs780094 polymorphism is associated with susceptibility of type 2 diabetes, reduced fasting plasma glucose levels, increased triglycerides levels and lower HOMA-IR in Japanese population. *J. Hum. Genet.* **55**, 600-604 (2010).
30. Kathiresan, S., Willer, C. J., Peloso, G. M., Demissie, S., Musunuru, K., Schadt, E. E. *et al.* Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat. Genet.* **41**, 56-65 (2009).
31. Takeuchi, F., Yamamoto, K., Katsuya, T., Nabika, T., Sugiyama, T., Fujioka, A., *et al.* Association of genetic variants for susceptibility to obesity with type 2 diabetes in Japanese individuals. *Diabetologia* **54**, 1350-1359 (2011).

Table 1 Clinical characteristics of the subjects

	Case	Control	<i>P</i> -value
Gender (men/women)	594/502	182/399	<0.0001
Age (years)	53.8 ± 12.5	47.2 ± 14.8	<0.0001
Body mass index (kg m ⁻²)	32.0 ± 5.2	21.4 ± 2.1	<0.0001
Plasma glucose (mg per 100 ml)	131.9 ± 45.1	90.9 ± 7.7	<0.0001
Triglycerides (mg per 100 ml)	184.0 ± 145.3	76.0 ± 28.8	<0.0001
High-density lipoprotein cholesterol (mg per 100 ml)	51.3 ± 18.0	67.3 ± 14.0	<0.0001
Systolic blood pressure (mm Hg)	139.9 ± 17.0	111.1 ± 10.2	<0.0001
Diastolic blood pressure (mm Hg)	85.3 ± 12.2	69.4 ± 7.5	<0.0001
Prevalence of metabolic disease			
Dyslipidemia	858 (78%)	0	
Hypertension	974 (89%)	0	
Impaired fasting glucose	770 (70%)	0	
No. of subjects under treatment			
Dyslipidemia	319	0	
Hypertension	466	0	
Diabetes	379	0	

Data are shown as the mean ± s.d. Men/women ratio was analyzed by the χ^2 -test. *P*-values for quantitative traits were analyzed using the Mann–Whitney *U*-test.

Table 2 Genotype and association tests

SNP ID	Nearby	Allele1/	Risk	Genotype		Model	P-value	OR (95% CI)
	gene	Allele2	allele	Case	Control			
rs3101336	<i>NEGR1</i>	A/G	G	4/153/938	1/88/492	a	0.55	1.09 (0.82 – 1.46)
rs2568958	<i>NEGR1</i>	A/G	A	932/154/4	490/88/1	a	0.60	1.08 (0.81 – 1.44)
rs2815752	<i>NEGR1</i>	A/G	A	938/154/4	492/88/1	a	0.58	1.09 (0.81 – 1.45)
rs10913469	<i>SEC16B</i>	T/C	C	596/430/69	331/224/26	a	0.054	1.19 (1.00 – 1.43)
rs6548238	<i>TMEM18</i>	T/C	C	8/169/919	7/101/473	a	0.037	1.32 (1.02 – 1.71)
rs7561317	<i>TMEM18</i>	G/A	G	917/171/8	472/102/7	a	0.037	1.32 (1.02 – 1.71)
rs7647305	<i>ETV5</i>	C/T	C	1015/77/0	537/39/1	a	0.57	1.13 (0.75 – 1.70)
rs10938397	<i>GNPDA2</i>	A/G	G	513/470/111	289/235/55	a	0.37	1.08(0.92 – 1.27)
rs6265	<i>BDNF</i>	A/G	G	182/522/388	100/283/196	a	0.29	1.09 (0.93 – 1.26)
rs925946	<i>BDNF</i>	T/G	T	2/74/1020	0/44/537	a	0.75	0.94 (0.63 – 1.40)
rs10838738	<i>MTCH2</i>	G/A	G	95/437/564	67/234/277	a	0.023	0.83 (0.71 – 0.98)
rs7498665	<i>SH2B1</i>	G/A	G	30/280/784	9/133/439	a	0.045	1.25(1.01 – 1.55)
rs9939609	<i>FTO</i>	T/A	A	650/379/65	389/170/20	a	0.00013	1.45 (1.20 – 1.75)
rs8050136	<i>FTO</i>	C/A	A	650/377/65	389/171/19	a	0.00011	1.45 (1.20 – 1.76)
rs6499640	<i>FTO</i>	A/G	A	33/248/814	9/134/438	a	0.35	1.11 (0.89 – 1.37)
rs1121980	<i>FTO</i>	A/G	A	74/404/614	25/196/357	a	0.0028	1.32 (1.10 – 1.58)
rs1558902	<i>FTO</i>	A/T	A	63/381/648	19/170/391	a	6.6×10^{-5}	1.47 (1.22 – 1.78)
rs1421085	<i>FTO</i>	C/T	C	63/380/648	19/170/390	a	7.4×10^{-5}	1.47 (1.21 – 1.78)
rs1424233	<i>MAF</i>	C/G	C	644/368/83	329/211/40	a	0.63	1.04 (0.88 – 1.23)
rs17782313	<i>MC4R</i>	T/C	C	638/392/66	348/200/33	a	0.25	1.11 (0.93 – 1.32)
rs12970134	<i>MC4R</i>	A/G	A	21/304/762	16/157/404	a	0.68	1.04 (0.85 – 1.28)
rs489693	<i>MC4R</i>	C/A	A	682/367/45	365/191/24	a	0.43	1.08 (0.89 – 1.30)
rs17700144	<i>MC4R</i>	A/G	A	2/67/1025	1/31/546	a	0.21	1.32 (0.85 – 2.05)
rs29941	<i>KCTD15</i>	T/C	C	672/378/45	349/209/22	a	0.62	1.05 (0.87 – 1.26)
rs11084753	<i>KCTD15</i>	G/A	G	96/472/528	56/243/282	a	0.60	1.04 (0.89 – 1.23)
rs3764220	<i>SCG3</i>	A/G	A	844/240/11	438/120/20	d	0.0010	3.74 (1.70 – 8.23)
rs2293855	<i>MTMR9</i>	A/G	A	482/466/146	215/282/83	r	0.0015	1.43 (1.15 – 1.78)
rs987237	<i>TFAP2B</i>	A/G	G	664/382/48	363/188/27	a	0.48	1.07 (0.89 – 1.28)
rs7826222	<i>MSRA</i>	G/C	C	423/511/162	232/269/79	a	0.66	1.04 (0.89 – 1.21)
rs2605100	<i>LYPLAL1</i>	A/G	G	39/315/740	19/159/399	a	0.67	0.96 (0.79 – 1.17)
rs780094	<i>GCKR</i>	T/C	C	213/510/368	124/287/169	a	0.17	1.11 (0.96 – 1.28)
rs1260326	<i>GCKR</i>	T/C	T	364/519/211	160/297/124	a	0.072	1.15 (0.99 – 1.33)
rs174547	<i>FADS1</i>	T/C	C	426/537/133	232/262/87	a	0.48	0.95 (0.81 – 1.11)

The OR for each SNP was adjusted simultaneously for age and gender.

Table 3 Results for SNP pairs for epistasis in case-control analysis

SNP1	SNP2	OR (95% CI)	Epistasis <i>P</i> -value
rs2293855	rs3764220	0.07 (0.01 – 0.70)	0.023
rs2293855	rs1421085	0.80 (0.55 – 1.18)	0.27
rs2293855	rs1558902	0.81 (0.55 – 1.19)	0.29
rs2293855	rs8050136	0.84 (0.57 – 1.24)	0.38
rs3764220	rs9939609	0.82 (0.56 – 1.21)	0.32
rs3764220	rs1421085	1.60 (0.44 – 5.82)	0.48
rs3764220	rs1558902	1.60 (0.44 – 5.84)	0.47
rs3764220	rs8050136	1.58 (0.43 – 5.74)	0.49
rs3764220	rs9939609	1.57 (0.43 – 5.71)	0.49

Table 4 Genotype and association tests of dyslipidemia, hypertension, and impaired fasting glucose

	Control		Dyslipidemia (n=858)		Hypertension (n=974)			Impaired fasting glucose (n=770)		
	genotype	Genotype	<i>P</i> -value	OR (95% CI)	Genotype	<i>P</i> -value	OR (95% CI)	Genotype	<i>P</i> -value	OR (95% CI)
rs9939609	389/170/20	511/297/48	0.00051	1.42 (1.17 – 1.73)	575/342/57	9.5×10 ⁻⁵	1.47 (1.21 – 1.79)	458/266/44	0.00024	1.46 (1.19 – 1.79)
rs8050136	389/171/19	511/295/48	0.00045	1.43 (1.17 – 1.74)	575/339/57	8.9×10 ⁻⁵	1.48 (1.22 – 1.80)	458/265/44	0.00020	1.47 (1.20 – 1.80)
rs1558902	19/170/391	47/298/510	0.00024	1.45 (1.19 – 1.77)	55/344/573	4.8×10 ⁻⁵	1.50 (1.23 – 1.83)	43/268/456	0.00011	1.49 (1.22 – 1.83)
rs1421085	19/170/390	47/297/509	0.00026	1.45 (1.19 – 1.77)	55/343/573	5.4×10 ⁻⁵	1.50 (1.23 – 1.82)	43/267/456	0.00012	1.49 (1.22 – 1.83)
rs3764220	438/120/20	658/190/9	0.0042	3.37 (1.47 – 7.76)	752/210/11	0.0028	3.34 (1.52 – 7.38)	592/170/8	0.0026	3.81 (1.60 – 9.10)
rs2293855	215/282/83	366/372/118	0.0098	1.35 (1.08 – 1.70)	430/415/127	0.00098	1.46 (1.17 – 1.83)	351/315/104	0.00039	1.53 (1.21 – 1.93)

The OR for each SNP was adjusted simultaneously for age and gender.

Supplementary Table 1 The power of the test

MAF	OR			
	1.2	1.3	1.4	1.5
0.1	0.017	0.051	0.123	0.240
0.2	0.040	0.136	0.317	0.546
0.3	0.062	0.211	0.457	0.708
0.4	0.076	0.256	0.527	0.771
0.5	0.081	0.267	0.538	0.776